VIP Structure Elucidation

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Isolation, Structure Elucidation, and Biomimetic Total Synthesis of Versicolamide B, and the Isolation of Antipodal (−)-Stephacidin A and (+)-Notoamide B from *Aspergillus versicolor* NRRL 35600**

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Prenylated indole alkaloids containing the bicyclo[2.2.2]diazaoctane ring system as a core structure, now number more than 38 family members. These natural substances, produced by various genera of fungi, in particular *Aspergillus* and *Penicillium* spp. (among others), exhibit a range of interesting structural and stereochemical features. Significantly, a myriad of biological activities, including insecticidal, antitumor, anthelmintic, calmodulin inhibitory, and antibacterial properties, are displayed by members of this family. Structurally, these substances arise from the oxidative condensation of one or two isoprene units, tryptophan, and another cyclic amino acid residue such as proline, β-methylproline, or pipecolic acid. With respect to the relative stereochemistry within the core bicyclo[2.2.2]diazaoctane ring system, all of the known members of the paraherquamide family, for example, paraherquamides (**1** and **2**), stephacidins (**3** and **4**), and notoamides (**5** and **6**) possess a *syn* configuration, while only the brevianamides (**9** and **10**) possess the *anti* relative configuration (Scheme 1). The *syn*/*anti* relationship refers to the relative configuration between the C19–C22 bond (sclerotiamide numbering) and the C17–N13 bond of the cyclic amino acid residue (proline, β-methylproline, or pipecolic acid; Scheme 2). This relationship reveals that to construct the core ring system biosynthetically in the oxidative cyclization process(es) both faces of the isoprene-derived dienophile participate in the ring-forming process. However,

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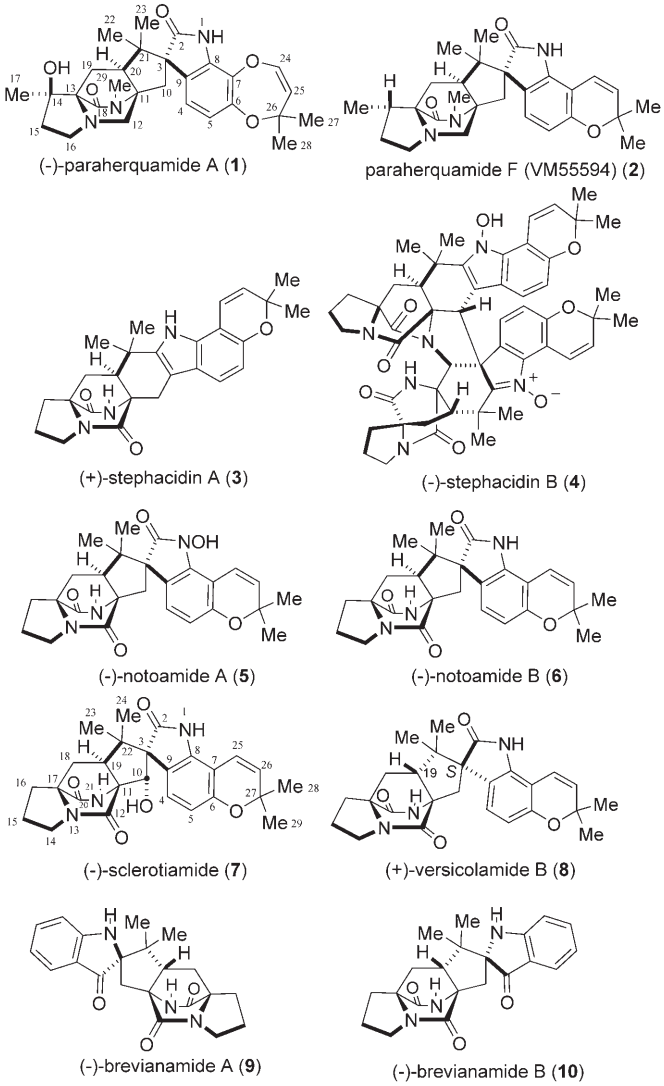
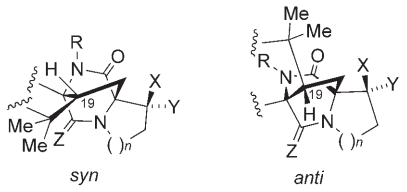
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Structures of several members of the paraherquamide/stephacidin/brevianamide family of prenylated indole alkaloids.



Scheme 2. *Syn* and *anti* relative configurations at C19 of the bicyclo-[2.2.2]diazaoctane ring system (sclerotiamide numbering).

until now this stereochemical divergence was cleanly separated between the brevianamides and all other members of this growing family of natural products. Herein, we describe the isolation, structure elucidation, and confirmatory biomimetic total synthesis of the first member of the paraherquamide/stephacidin family to possess the rare *anti* relative configuration within the bicyclo[2.2.2]diazaoctane ring system. We propose the new name of versicolamide B for this natural product (**8**), which is a minor metabolite of *Aspergillus versicolor* NRRL 35600. We have assigned the absolute configuration to this compound on the basis of the circular dichroism (CD) spectra, and have concluded that it possesses the *ent* configuration with respect to the bicyclo-[2.2.2]diazaoctane core. Surprisingly, and just as striking, we have also isolated (−)-stephacidin A and (+)-notoamide B from *Aspergillus versicolor* NRRL 35600 and conclude that these natural products are produced as the corresponding enantiomers to the structures that have been previously described. The provocative biogenetic implications of these stereochemical findings are discussed herein.

Previous studies from our research group,[1] as well as those from the groups of Birch[2] and Sammes,[3] suggest that the bicyclo[2.2.2]diazaoctane core of these alkaloids likely arises in nature through a biosynthetic intramolecular Diels–Alder (IMDA) construction. We have recently completed the total synthesis of several prenylated indole alkaloids containing the common bicyclo[2.2.2]diazaoctane ring system by using biogenetically inspired IMDA cycloaddition reactions.[1a,4] The synthesized compounds include brevianamide B (**10**), stephacidin A (**3**), marcfortine C, and the recently discovered fungal metabolite notoamide B (**6**; Scheme 1).[4,5] We demonstrate herein that this general strategy was easily amendable to the total synthesis of versicolamide B and provided unambiguous structural and relative stereochemical corroboration for this stereochemically unique natural product.

The isolate of *A. versicolor* was obtained from a basidioma of *Gandoderma australe* collected in a Hawaiian forest, and was selected for investigation as part of a project targeting mycoparasitic and fungicolous fungal isolates as sources of new bioactive natural products.[6] This isolate was cultured by solid-substrate fermentation on rice, and the crude extract of these cultures showed significant anti-insectan activity. Five "known" compounds (sterigmatocystin, brevianamide F, stephacidin A, norgeamide D, and notoamide B) were obtained from this extract. The structures of four of the known compounds were confirmed by comparison of their NMR and MS data with literature values,[7] while the structure of notoamide B was independently assigned because the report describing this metabolite[5] had not yet appeared in the literature. Sterigmatocystin was a major component, and appeared to be responsible for most of the anti-insectan activity of the crude extract. The molecular formula of an additional minor component (versicolamide B; **8**) was established as $C_{26}H_{29}N_3O_4$ (14 degrees of unsaturation) on the basis of NMR and HRMS (ESI) data (see the Supporting Information for a detailed NMR spectroscopy analysis).

Circular dichroism spectroscopy has been used as a method to assign the absolute configuration of spiro-oxindole alkaloids.[9] The Cotton effect at 250–350 nm is considered to be an indication of the configuration at spiro stereogenic center C3.[9a,b] The CD spectra of **8** and the (+)-notoamide B isolated from *A. versicolor* both show a positive Cotton effect in the spiro-oxindole absorbance region around 280 nm (Figure 1), which suggests the same 3*S* configuration for each compound, and is in agreement with the configuration previously assigned for synthetic *ent*-(+)-paraherquamide B. Correspondingly, the absolute configuration of **8** is proposed as shown (Scheme 1).

Of further interest was the surprising observation that the (−)-stephacidin A and (+)-notoamide B samples isolated from *A. versicolor* possess the opposite absolute configurations to those previously reported (Figure 1).[5,7c] These assignments were based on examination of the CD spectra and the optical rotation values for these substances.

In their elegant total synthesis of stephacidin A, Baran et al. previously reported the optical rotation values and CD spectra for natural (+)-stephacidin A (obtained from Professor Fenical's research group) as well as corroborating data on synthetic (+)-stephacidin A.[10a] In addition, these researchers recorded mirror-image CD spectra for natural (+)-stephacidin A and synthetic (−)-stephacidin A.[10c]

The optical rotation values for the natural samples derived from *Aspergillus versicolor* NRRL 35600 used in this study are as follows: (+)-versicolamide B $[\alpha]_D = +26\ \mathrm{deg\,cm^3\,g^{-1}\,dm^{-1}}$ ($c = 0.1\ \mathrm{g\,cm^{-3}}$, acetone); *ent*-stephacidin A $[\alpha]_D = -32\ \mathrm{deg\,cm^3\,g^{-1}\,dm^{-1}}$ ($c = 0.05\ \mathrm{g\,cm^{-3}}$, CH_2Cl_2/MeOH; 1:1), lit. $[\alpha]_D = +61.5\ \mathrm{deg\,cm^3\,g^{-1}\,dm^{-1}}$ ($c = 0.26\ \mathrm{g\,cm^{-3}}$, 1:1 CH_2Cl_2/MeOH);[10] *ent*-notoamide B $[\alpha]_D = +102\ \mathrm{deg\,cm^3\,g^{-1}\,dm^{-1}}$ ($c = 0.05\ \mathrm{g\,cm^{-3}}$ MeOH), lit. $[\alpha]_D = -118\ \mathrm{deg\,cm^3\,g^{-1}\,dm^{-1}}$ ($c = 0.064\ \mathrm{g\,cm^{-3}}$, MeOH).[5] We have further determined that the (−)-stephacidin A sample collected from *A. versicolor* is optically pure by HPLC on a chiral stationary phase (see the Supporting Information). These data, along with the CD spectra, rigorously support the surprising fact that *Aspergillus versicolor* NRRL 35600 produces the opposite enantiomers of stephacidin A and notoamide B to those obtained from the related fungi *Aspergillus ochraceus* WC76466 and from the marine-derived *Aspergillus* sp., which were studied by Tsukamoto and co-workers.[5]

The structure and relative configuration of versicolamide B were corroborated through a biomimetic, racemic total synthesis. Our synthesis of **8** commenced with a Mitsonobu-type elimination (PBu_3, DEAD) of the recently prepared alcohol **11**[4] to afford an intermediate enamide, which was then treated with Me_3OBF_4 and Cs_2CO_3 to cleanly provide the desired lactim ether **12** in good yield (Scheme 3).

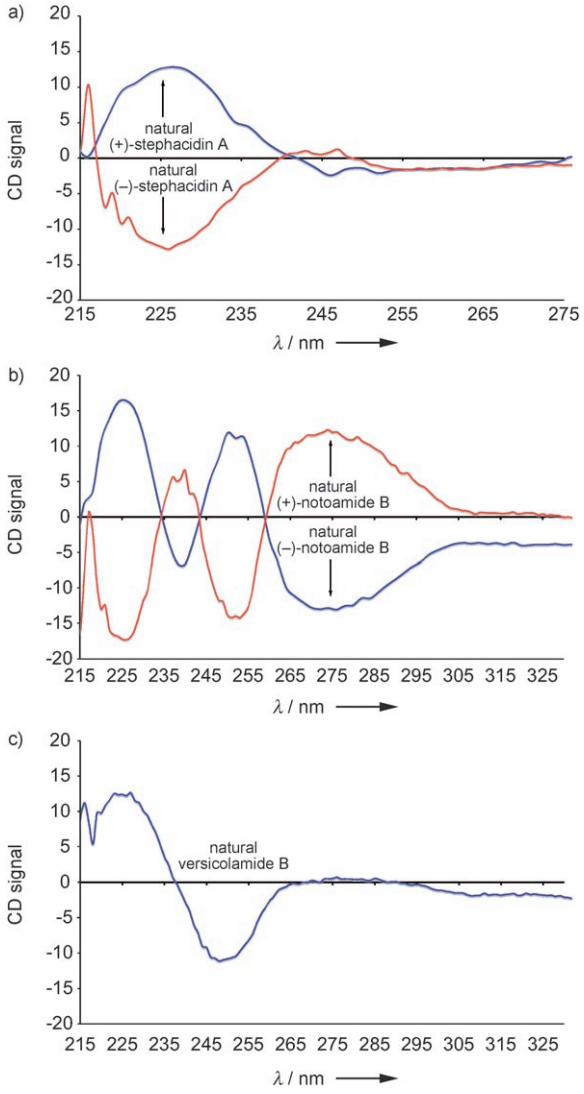
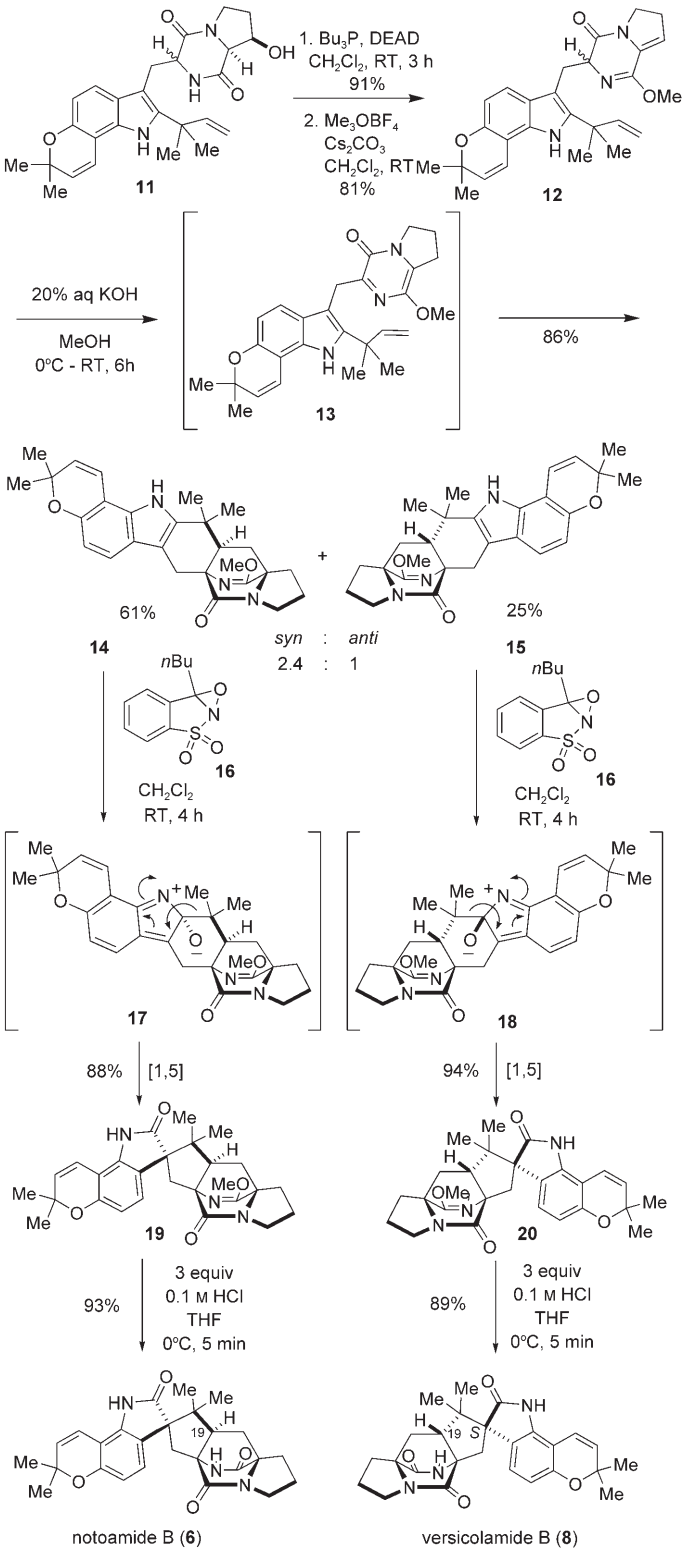


Figure 1. CD spectra of *Aspergillus versicolor* NRRL 35600 metabolites: a) *ent*-(−)-stephacidin A; b) *ent*-(+)-notoamide B; c) versicolamide B. All CD spectra were recorded in 1:1 CH_2Cl_2/MeOH.



Scheme 3. Synthesis of (±)-**6** and (±)-**8**. Structures are depicted with the correct relative and absolute configuration for the natural materials isolated from *Aspergillus versicolor* NRRL 35600; all substances after **11** were produced in racemic form. DEAD = diethyl azodicarboxylate.

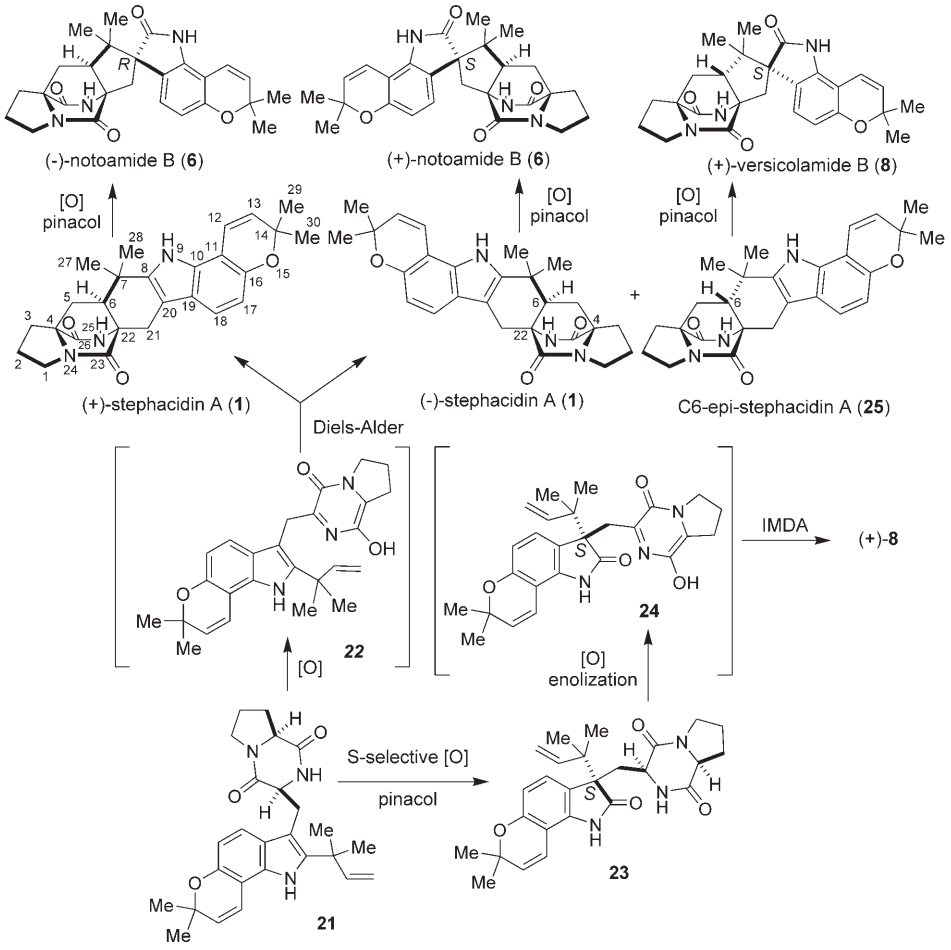
With lactim ether **12** in hand, we were ready to try the key biomimetic cycloaddition reaction. As recently reported, treatment of **12** with 20% aqueous KOH in MeOH (0°C–RT, 6 h) effected tautomerization to the intermediate azadiene **13**, which spontaneously underwent IMDA cycloaddition to produce cycloadducts **14** and **15** as a 2.4:1 mixture of diastereomers in favor of the *syn* stereoisomer. Interestingly, the intermediate **13** is a metastable substance that could be observed by both thin-layer chromatography (TLC) and ^{1}H NMR spectroscopy analysis. As observed by TLC analysis during the reaction, lactim ether **12** ($R_f = 0.75$, EtOAc) disappeared within 1.5 h and azadiene **13** ($R_f = 0.25$, EtOAc) appeared. This TLC spot then slowly disappeared and gave rise to cycloadducts **14** and **15** ($R_f \approx 0.4$, EtOAc). The intermediate **13** was also observed by ^{1}H NMR spectroscopy through treatment of **12** with KOD in CD_3OD/D_2O, which was carried out in an NMR tube.

The tentative stereochemical assignments for cycloadducts **14** and **15** were confirmed upon their transformation into (±)-notoamide B (**6**) and (±)-versicolamide B (**8**), respectively. We recently reported that **6** could be prepared

through cleavage of the lactim ether *syn*-cycloadduct **14** to give stephacidin A, which was then subjected to a stereoselective oxidation and pinacol-type rearrangement to cleanly produce notoamide B (**6**).[4] However, this protocol proved problematic for the completion of the synthesis of (±)-versicolamide B (**8**) from the corresponding *anti*-cycloadduct **15**. The intermediate indole derived from cleavage of the lactim ether of **15** was found to be unstable when exposed to the atmosphere and underwent a facile ring-opening/hydrolysis of the diketopiperazine to produce the corresponding amino acid.

We therefore decided to perform the oxidation/pinacol rearrangement to give the spiro-oxindole prior to cleavage of the methyl lactim ether. Indeed, we were pleased to find that treatment of cycloadducts **14** and **15** with excess oxaziridine **16**[11] in CH_2Cl_2 cleanly provided the desired spiro-oxindoles **19** and **20**, respectively (Scheme 3). The stereochemical result of these oxidative rearrangements can be rationalized by considering that epoxidation of the 2,3-disubstituted indoles **14** and **15** occurs from the less hindered α face, followed by ring opening of the incipient epoxides to their respective 2-alkoxyindole intermediates **17** and **18**. A subsequent α-face ring contraction by a [1,5] sigmatropic shift successfully furnished **19** and **20**, each as a single diastereomer. Finally, the lactim ethers of both **19** and **20** were uneventfully cleaved by treatment with 0.1M HCl (3 equiv) in THF (0°C, 5 min) to successfully provide (±)-notoamide B (**6**) and (±)-versicolamide B (**8**), respectively. The biomimetic synthesis of (±)-versicolamide B was thus completed in 18 steps and 1.8% overall yield, and that of (±)-notoamide B was completed in 18 steps and 4.2% overall yield (both from commercially available 6-hydroxyindole). All the ^1H and ^{13}C NMR spectroscopic data were identical to those of natural **6** and **8**, thus corroborating the relative stereochemical assignment based on NMR spectroscopy as discussed above.

The discovery of (+)-versicolamide B and the co-occurring metabolites (−)-stephacidin A and (+)-notoamide B adds another intriguing twist to the emerging picture of the biogenesis of these alkaloids. Several possible biosynthetic relationships are depicted in Scheme 4 and from this viewpoint, notoamide E (**21**) is envisioned to be the key biosynthetic progenitor.[12] Oxidation and tautomerization of **21** would yield the key achiral azadiene species **22** that can, in principle, undergo cycloaddition to produce four stereoisomers: stephacidin A (as the (+) and (−) enantiomers) and C6-*epi*-stephacidin A (**25**; also as the (+) and (−) enantiomers; only one is shown). Oxidation of the 2,3-indolic moiety of (−)-stephacidin A to the corresponding spiro-oxindole produces (+)-notoamide B. Similar face-selective oxidation of C6-*epi*-stephacidin A (**25**) would produce (+)-versicolamide B. This reasonable biogenetic relationship also implies that C6-*epi*-stephacidin A (**25**) may be an as yet undetected natural product produced by *A. versicolor* with the absolute configuration shown in Scheme 4. What is most curious is the enantiofacial divergence of this presumed cycloaddition throughout the different species of *Aspergillus* that have been demonstrated to produce stephacidin A. Thus far, (+)-stephacidin A is the enantiomer produced in *Aspergillus ochraceus* WC76466[7c] and the marine-derived *Aspergillus* sp. described by Tsukamoto and co-workers.[5] The occurrence of (−)-stephacidin A and its presumed oxidation metabolite (+)-notoamide B in the same stereochemical series, but distinct from that reported from these other *Aspergillus* isolates, is striking. If the proposed biogenesis based on IMDA construction is correct, this would mandate that each fungal species evolved a means by which to select for the production of one enantiomeric cycloadduct (that is, (+)- or (−)-stephacidin A). This selection could be either



Scheme 4. Possible biosynthetic relationships of some prenylated indole alkaloids.

through manipulation of the precyclization conformers of putative azadiene **22**, or perhaps through selective catabolism of one enantiomer from an initially produced racemate. The latter seems less plausible as we have confirmed the optical purity of the (−)-stephacidin A herein and Baran et al. have previously confirmed the optical purity of natural (+)-stephacidin A.[10] The occurrence of (+)-versicolamide B as a co-metabolite with (−)-stephacidin A and (+)-notoamide B is even more perplexing, since the bicyclo[2.2.2]diazaoctane core of this metabolite is pseudoenantiomeric to that of these co-metabolites (but corresponds to that of (+)-stephacidin A). Does this choice occur through the selection of two of the four possible transition states accessible to **22**?

Alternatively, notoamide E (**21**) could be oxidized by an *S*-selective indole oxidase to give a diastereomer (**23**) of the natural oxindole species notoamide C[5]. Further oxidation and enolization of **23** could provide azadiene **24**, which could undergo IMDA cycloaddition to directly furnish (+)-versicolamide (**8**). Ab initio calculations suggest,[13] and experimental model studies support,[14] that the intrinsic facial bias of azadiene species similar to **24** result in them having a strong proclivity to form the *anti* relative configuration at C19 present in versicolamide B. We are currently preparing isotopically labeled substrates, including of **21** and **23**, to further investigate this fascinating stereochemical paradox.

In summary, the first member of the larger paraherquamide family of prenylated indole alkaloids that contains the *anti* relative configuration at C19 in the bicyclo-[2.2.2]diazaoctane ring system has been isolated and its structure, named versicolamide B, rigorously elucidated through spectroscopic means and biomimetic total synthesis.

The production of this metabolite by *A. versicolor* suggests that the putative biosynthetic IMDA construction, which leads to the major metabolites within the producing organism, may suffer some stereochemical "leakage" with respect to the facial selectivity of addition to the reverse isoprene moiety anchored at the indole 2-position. Assuming that the biosynthesis of the distinct enantiomers of (+)- and (−)-stephacidin A, and the presumed further oxidation products (−)- and (+)-notoamide B, respectively, proceed through stereochemically parallel pathways in the respective *Aspergillus* species, it is fascinating that each organism must contain specific *R*- and *S*-selective indole oxidases paired to their respective stephacidin A enantiomer. Based on our observations, it is reasonable to anticipate that stereochemically related members of this family may be produced by other fungi, albeit in trace amounts. The surprising stereochemical paradox posed by the existence of (+)-versicolamide B[15] along with the opposite enantiomers of stephacidin A and notoamide B demand explanation on biogenetic grounds and constitute a major thrust of our ongoing work. Studies to further establish the relationship of these and simpler precursor metabolites in the biosynthesis of this family of agents, as well as efforts to carefully study the metabolite profile of *A. versicolor*, are currently under investigation.

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[1] a) R. M. Williams, R. J. Cox, *Acc. Chem. Res.* **2003**, *36*, 127; b) R. M. Williams, *Chem. Pharm. Bull.* **2002**, *50*, 711; c) R. M. Williams, E. M. Stocking, J. F. Sanz-Cevera, *Top. Curr. Chem.* **2000**, *209*, 98.

[2] J. Baldas, A. J. Birch, R. A. Russell, *J. Chem. Soc. Perkin Trans. 1* **1974**, 50.

[3] A. E. A. Porter, P. G. Sammes, *Chem. Commun.* **1970**, 1103.

[4] a) T. J. Greshock, A. W. Grubbs, S. Tsukamoto, R. M. Williams, *Angew. Chem.* **2007**, *119*, 2312; *Angew. Chem. Int. Ed.* **2007**, *46*, 2262; b) A. W. Grubbs, G. D. Artman III, S. Tsukamoto, R. M. Williams, *Angew. Chem.* **2007**, *119*, 2307; *Angew. Chem. Int. Ed.* **2007**, *46*, 2257.

[5] H. Kato, T. Yoshida, T. Tokue, Y. Nojiri, H. Hirota, T. Ohta, R. M. Williams, S. Tsukamoto, *Angew. Chem.* **2007**, *119*, 2304; *Angew. Chem. Int. Ed.* **2007**, *46*, 2254.

[6] a) S. T. Deyrup, D. C. Swenson, J. B. Gloer, D. T. Wicklow, *J. Nat. Prod.* **2006**, *69*, 608; b) S. H. Shim, D. C. Swenson, J. B. Gloer, P. F. Dowd, D. T. Wicklow, *Org. Lett.* **2006**, *8*, 1225; c) S. V. Mudur, J. B. Gloer, D. T. Wicklow, *J. Antibiot.* **2006**, *59*, 500.

[7] a) R. J. Cole, M. A. Schweikert, *Handbook of Secondary Fungal Metabolites*, *Vol. 1*, Academic Press, New York, **2003**, pp. 597–598; b) A. W. Grubbs, G. D. Artman, R. M. Williams, *Tetrahedron Lett.* **2005**, *46*, 9013; c) J. Qian-Cutrone, S. Huang, Y. Shu, D. Vyas, C. Fairchild, A. Menendez, K. Krampitz, R. Dalterio, S. E. Klohr, Q. Gao, *J. Am. Chem. Soc.* **2002**, *124*, 14556; d) A. J. Birch, R. A. Russell, *Tetrahedron* **1972**, *28*, 2999.

[8] a) A. C. Whyte, J. B. Gloer, D. T. Wicklow, P. F. Dowd, *J. Nat. Prod.* **1996**, *59*, 1093; b) M. Yamazaki, E. Okuyama, M. Kobayashi, H. Inoue, *Tetrahedron Lett.* **1981**, *22*, 135.

[9] a) H. Takayama, T. Shimizu, H. Sada, Y. Harada, M. Kitajima, N. Aimi, *Tetrahedron* **1999**, *55*, 6841; b) T. Mugishima, M. Tsuda, Y. Kasai, H. Ishiyama, E. Fukushi, J. Kawabata, M. Watanabe, K. Akao, J. Kobayashi, *J. Org. Chem.* **2005**, *70*, 9430; c) T. D. Cushing, J. F. Sanz-Cervera, R. M. Williams, *J. Am. Chem. Soc.* **1996**, *118*, 557.

[10] a) P. S. Baran, C. A. Guerrero, N. B. Ambhaikar, B. D. Hafensteiner, *Angew. Chem.* **2005**, *117*, 612; *Angew. Chem. Int. Ed.* **2005**, *44*, 606; b) P. S. Baran, C. A. Guerrero, B. D. Hafensteiner, N. A. Ambhaikar, *Angew. Chem.* **2005**, *117*, 3960; *Angew. Chem. Int. Ed.* **2005**, *44*, 3892; c) P. S. Baran, B. D. Hafensteiner, N. B. Ambhaikar, C. A. Guerrero, J. D. Gallagher, *J. Am. Chem. Soc.* **2006**, *128*, 8678.

[11] a) F. A. Davis, J. C. Townson, D. B. Vashi, R. ThimmaReady, J. P. McCauley, M. E. Harakal, D. J. Gosciniak, *J. Org. Chem.* **1990**, *55*, 1254; b) B. B. Snider, H. Zeng, *J. Org. Chem.* **2003**, *68*, 545.

[12] Notoamide E has recently been verified as a natural metabolite of the marine-derived *Aspergillus* spp. by comparison to an authentic, synthetic sample provided by this research group, see Ref. [4b] (S. Tsukamoto, personal communication); to be reported elsewhere.

[13] a) L. R. Domingo, J. F. Sanz-Cervera, R. M. Williams, M. T. Picher, J. A. Marco, *J. Org. Chem.* **1997**, *62*, 1662; b) L. R. Domingo, R. J. Zaragozá, R. M. Williams, *J. Org. Chem.* **2003**, *68*, 2895.

[14] a) L. A. Adams, C. R. Gray, R. M. Williams, *Tetrahedron Lett.* **2004**, *45*, 4489; b) L. A. Adams, M. W. N. Valente, R. M. Williams, *Tetrahedron* **2006**, *62*, 5195.

[15] We chose the descriptor versicolamide "B" with the anticipation that the, as yet, undiscovered "A" series would come from β-methylproline in paraherquamide-producing fungi. Efforts to detect such potential metabolites are currently underway.

